

Hyseq, Inc.

## Prototype DNA Diagnostic Chip to Sequence Entire Genes

*Since 1990, scientists have heard the same refrain: DNA-based diagnostics could render "modern medicine" obsolete. Preventive healthcare that would preclude the onset of symptoms from genetic disorders, or would mitigate their effects, could begin at birth. Customized treatments could be used to treat the diseases that do manifest. However, before the new wave of customized, preventative, and post-manifestation treatments could mature and become the norm, physicians needed quick and affordable access to a full map of the patient's genetic code. As of 1995, available DNA diagnostics techniques were too expensive, time-consuming, and inaccurate to effect such a change in modern medicine. At the time, existing diagnostic chips cost thousands of dollars and did not have enough probes to test an entire strand of DNA to fully detect and understand mutations.*

*In 1994, Hyseq, Inc. was formed to develop the techniques critical for an instrument capable of quickly, inexpensively, and automatically sequencing entire genes using libraries of short DNA probes that hybridize in an overlapping fashion with the target DNA to enable full sequencing. The unique feature of this system is that it is designed to sequence DNA that had not been sequenced before. Other chips were capable of operating only with DNA whose sequence was already known. Hyseq sought venture capitalist funding; however, the funding sources wanted a 70-percent ownership in the company due to the project's extremely high level of risk. In order to help fund this novel initiative for de novo sequencing without giving a significant share of the business to sources of private capital, Hyseq sought cost-shared funding through the Advanced Technology Program (ATP).*

*In January 1995, ATP awarded Hyseq \$2 million for a two-year project. The ATP project was a scientific success. By late 1997, Hyseq had developed a prototype diagnostic chip that could affordably sequence DNA strands five times as long as any other chip on the market, to greater than 99.99-percent accuracy. While post-project business considerations delayed commercialization, Hyseq's post-project partner, Applied Biosystems, has pledged full support for using Hyseq's chip to develop drugs to treat genetic disorders. In 2001, Hyseq and Applied Biosystems also spun off a majority-owned subsidiary, Callida Genomics, Inc., to pursue the DNA sequencing upon which Hyseq was founded. If Callida is a success, the company could bring new drugs and sequencing methods to market in the next 10 years (the normal drug development timeframe). Even if no drugs result, the ATP-funded project assisted development of a novel method of gene sequencing, resulted in a number of patents, and accelerated screening for new drugs.*

### COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

\* \* \*

Research and data for Status Report 94-05-0018 were collected during October - December 2001.

### DNA Diagnostics Are Time-Consuming and Expensive

In 1995, traditional DNA (deoxyribonucleic acid) sequencing and diagnostics had two steps, consisting of sample preparation and actual sequencing work.

Interpretation of the DNA sequences was then used in diagnostics. The sample preparation step required producing DNA strands that are either broken-up in a predictable pattern with enzymes or are identified through a reaction that locates specific genetic defects.

Sequencing is done by using a different enzyme reaction that makes a large set of incomplete copies of the DNA. Each of the incomplete DNA copies gets a tag at its end that is used to identify the last base of its DNA strand. This enzyme reaction produces a full set of shortened DNA segments whose length matches each step of the original DNA ladder. The strands are then separated by size using a process called gel electrophoresis. Since the strands naturally carry an electric charge, they move with the current through the gel. Longer strands take more time to worm their way through the gel. It is then possible to "read" the DNA sequence by identifying the tags at the end of the next DNA segment.

Unfortunately, these steps were time-consuming and expensive and required elaborate instrumentation. A new and improved approach was needed to generate the next wave of DNA technologies.

### **Hyseq's Chip Could Eventually Sequence Entire Genes**

The theory behind Hyseq's research efforts was that an entire DNA gene can be sequenced by using a set of short probes composed of every possible DNA sequence. Using the probes in parallel creates a quick and inexpensive process that eliminates many of the obstacles of the conventional process. With those obstacles removed, the separated DNA can then be further examined by sequencing. Small DNA segments are removed from the gel and separately placed onto a test chip covered with probes. The DNA binds to probes on the test chip in a pattern that is then used to read the sequence of the DNA segment. If similar DNA segments with an abnormal or different sequence were tested, it would produce a different binding pattern, leading to the exact identification of the changes in sequence.

Rather than using the industry-standard probe length that requires a million diagnostic probes to analyze a gene, Hyseq scientists developed two sets of 1,000 probes joined together with a ligase enzyme. These sets of probes could detect all possible five nucleotide combinations in parallel. In Hyseq's process, ligase allows the parallel processing of multiple strands of DNA, rather than the one-section-at-a-time serial processing of traditional DNA diagnostic machines.

Hyseq's goal was to develop a prototype sequencing chip that could read and sequence an entire gene. Parallel sequencing was not an industry norm, and Hyseq's chip required hundreds of thousands of chemical and biological processes to generate a successful sequence. This technology was remarkably forward-looking, but presented substantial scientific and business risks.

### **Scientists Set the Stage for Pathbreaking Technology**

A group of veteran scientists from the Department of Energy's Human Genome Project and the sequencing-by-hybridization (SBH) experiments in various national laboratories joined together to submit their ATP proposal offering to form a company, Hyseq, to pursue specific pathbreaking technology. From that proposal, ATP recognized that Hyseq had the potential to create technology that would take the first steps towards altering the course of diagnostic medicine. If successful, Hyseq's plan would enable a new generation of tools for gene sequencing and DNA diagnostic work. Further, this pathbreaking technology could potentially alter the way healthcare is administered, from birth to death, with the implementation of improved preventive care and personalized medication regimens. The proposed HyChip offered the potential to sequence the genetic codes of newborns to encourage a life-long preventive medicine approach that would allow longer and healthier lives, as well as customized medications to combat already-manifested illnesses in the most effective manner.

---

*The theory behind Hyseq's research efforts was that an entire DNA gene can be sequenced by using a set of short probes composed of every possible DNA sequence.*

---

The scientists sought private-sector funding before they formed the company in order to develop SBH processes with private equity. The few venture capitalists who considered funding such a risky endeavor demanded 70 percent of the company in return. Though this was commensurate with the risk of the company, the price was too high to entice these scientists away from their federally funded work with the

Human Genome Project. The \$2 million in cost-shared funds from ATP provided the impetus for them to move into the private sector to form Hyseq. The award, granted to a group of scientists who had not yet formed their company, was held by ATP and disbursed only after Hyseq became a legal entity and achieved an early proof-of-concept by accurately sequencing an industry-standard 64,000-nucleotide string. The ATP funds kept Hyseq's research and development operations going for its first few years.

Further, the award and its follow-on work attracted approximately \$10 million from other funding sources and convinced Hyseq to extend and expand research by 800 percent after the ATP-funded project ended in 1997. According to Mr. Deane Little, Director of Corporate Communications for Hyseq, "The ATP award kept the company going. Without the award, Hyseq never would have done the SBH platform" and never would have developed the HyChip.

---

***This technology was remarkably forward-looking, but presented substantial scientific and business risks.***

---

Spillover was an inevitable component of Hyseq's commercialization plan of forming joint production alliances. Throughout Hyseq's ATP-funded project, executives and scientists spoke at four conferences from 1995 to 1996 and issued a public press release in 1997. They received substantial publicity upon reporting at a biochip conference that they had scored on all one million possible 10-mer<sup>1</sup> probes on sequence samples for HIV and associated test controls. That was more than twice the largest number of probes previously scored by a chip on a sample. By the end of the ATP-funded project, Hyseq had also hired 19 new full-time-equivalent personnel. In addition, the company had filed for seven patents.

### **Post-Project Bottlenecks Delay Commercialization**

Once the HyChip's diagnostic probes were working properly, another problem surfaced. There were no

readers on the market that could process the HyChip's sequences. Hyseq could sequence DNA, but could not read the results or use them to conduct any diagnostic analysis. While large biotechnology companies had readers, they were expensive, incompatible with Hyseq's biochemical processes, and could not interface with Hyseq's database used to detect gene abnormalities. The company did not deal with this problem until after the close of the ATP-funded project. At that time, in 1997, Hyseq needed a partner to develop a HyChip-compatible reader and the diagnostic array technology. Thus, it entered into an agreement with PerkinElmer (now Applied Biosystems). Hyseq executives thought that this agreement would transform their start-up company into an industry powerhouse. The investment community lauded the PerkinElmer-Hyseq partnership and rewarded Hyseq with a successful \$44 million initial public offering during the fourth quarter of 1997.

However, early in the research phase, PerkinElmer redirected its focus away from the technology that had resulted from Hyseq's ATP project. Without the necessary R&D funds, Hyseq could not further develop its array technology. Ironically, a commercial reader compatible with the HyChip became available in late 1998, but Hyseq could not use the reader extensively because of the terms of its agreement with PerkinElmer.

To work around these difficulties, Hyseq used its market capital to fund research efforts to try to extend the HyChip's basic technology to other disciplines. Its efforts included experiments with biological bar codes, nanotechnology applications for diagnostic work, and agents to combat biological warfare. This search for new product lines continued for nearly two years until Applied Biosystems (the newly renamed bioscience section of PerkinElmer) again realized the potential for the HyChip and resumed funding product commercialization. Mr. Little commented that Applied Biosystems' refocused efforts on Hyseq's technology has altered the outlook of the company. In fact, the outlook is "significantly better than it was just a year ago."<sup>2</sup>

---

1. mer. This suffix is often used to indicate the number of nucleotides in an oligonucleotide.

2. Interview with Mr. Deane Little, Hyseq's Director of Corporate Communications, summer 2001.

## Hyseq Again On the Road to Changing Medicine

For the two years immediately following the conclusion of Hyseq's ATP award, use of the HyChip was limited to internal research. During that time, however, the HyChip achieved some remarkable successes. The chip sequenced the HIV virus correctly on all one million probes, achieved 100-percent accuracy on mitochondrial DNA tests, and sequenced 500 percent more bases than was possible with a traditional DNA diagnostic chip. The potential arising from these post-ATP tasks is far-reaching.

---

*ATP recognized that Hyseq had the potential to create technology that would take the first steps towards altering the course of diagnostic medicine.*

---

The HyChip enables researchers to generate sequences of, and develop other large data sets from, genes within a given species. Taken over a large population, this data can then be used to correlate genotype to phenotype (genetic code to physical traits). Once the database of genotype/phenotype correlations is completed for humans, the knowledge generated could usher in a new age of preventive healthcare, beginning with an individual genetic sequence performed immediately after birth. Working with Applied Biosystems, the HyChip can be used as part of the process to develop drugs both as preventative measures to keep genetic defects from developing into full-blown illnesses and also to treat those illnesses on a customized basis once they do manifest. Drug development is a long process that spans 8 to 10 years before a successful drug can reach the market. It is anticipated that in the next 10 to 15 years, the HyChip will help bring new drugs to market. As of 2001, Hyseq and Applied Biosystems had spun off a subsidiary, Callida Genomics, to pursue additional advances in DNA sequencing technology.

## Conclusion

ATP awarded Hyseq \$2 million to help develop a DNA diagnostic tool for sequencing entire genes at once. Hyseq succeeded in developing a prototype sequencing

chip, called the HyChip, during the ATP project. Shortly after the ATP project ended, however, an open-ended partnership agreement between Hyseq and Applied Biosystems resulted in a two-year delay of HyChip commercialization efforts. Fortunately, post-ATP project research and development and commercialization efforts are now going forward under a separate company, Callida Genomics, that was spun off from Hyseq and corporate partner Applied Biosystems. With Callida, the Hyseq ATP-funded technology is once again being utilized to promote advances in DNA diagnostics.



## PROJECT HIGHLIGHTS

### Hyseq, Inc.

**Project Title:** Prototype DNA Diagnostic Chip To Sequence Entire Genes (Sequencing By Hybridization Format 3 Megabase Diagnostics Instrumentation)

**Project:** To develop an instrument capable of quickly, inexpensively, and automatically sequencing entire genes using libraries of short DNA probes that hybridize in overlapping fashion with the target DNA to enable sequencing of an entire gene at once.

**Duration:** 1/1/1995-12/31/1997

**ATP Number:** 94-05-0018

#### Funding (in thousands):

ATP Final Cost	\$2,000	57%
Participant Final Cost	<u>1,498</u>	43%
Total	\$3,498	

**Accomplishments:** Using ATP funds, Hyseq developed a prototype called the HyChip that could sequence entire genes at one time, eliminating the need for many costly and time-consuming preparation steps.

The following are among HyChip's successes:

- Scored correctly on all one million probes with HIV sequence samples
- Scored 100-percent accuracy on mitochondrial DNA tests
- Sequenced 500 percent more bases with one chip than with traditional diagnostic chips

The accomplishments of this ATP-funded project led to the following patents:

- "Method of sequencing of genomes by hybridization of oligonucleotide probes"  
(No. 5,667,972: filed June 5, 1995, granted September 16, 1997)
- "Method of sequencing by hybridization of oligonucleotide probes"  
(No. 5,695,940: filed June 5, 1995, granted December 9, 1997)

- "Methods and compositions for detection or quantification of nucleic acid species"  
(No. 6,309,824: filed January 16, 1997, granted October 30, 2001)
- "Methods for sequencing repetitive sequences and for determining the order of sequence subfragments"  
(No. 6,297,006: filed March 4, 1997, granted March 4, 1997)
- "Method of sequencing of genomes by hybridization of oligonucleotide probes"  
(No. 6,018,041: filed July 29, 1997, granted January 25, 2000)
- "Methods and compositions for detection or quantification of nucleic acid species"  
(No. 6,383,742: filed August 15, 1997, granted May 7, 2002)
- "Reagent transfer device"  
(No. 5,882,930: filed November 10, 1997, granted March 16, 1999)
- "Reagent transfer device"  
(No. 6,255,119: filed September 25, 1998, granted July 3, 2001)

Knowledge spillover resulted through Hyseq's partnership with a university to conduct research and development, its joint venture with PerkinElmer, and presentations it made at biochip conferences.

**Commercialization Status:** Before the ATP project, Hyseq had no commercializable products, just a research idea. At project closeout, Hyseq had developed a prototype DNA diagnostic chip, called the HyChip, that could sequence an entire gene at once. Hyseq also had entered into an exclusive agreement with PerkinElmer, a major pharmaceutical company, to develop and commercialize the HyChip. Due to internal business decisions made several months after the end of Hyseq's ATP project, PerkinElmer changed course and did not fund the development of the HyChip. By 2000, PerkinElmer committed itself as a company to bioscience, renaming its bioscience division Applied Biosystems. As part of the bioscience focus, Applied Biosystems began using Hyseq's technology in its drug development efforts. In 2001, Applied Biosystems and Hyseq spun off a subsidiary, Callida Genomics. Callida, which inherited all the intellectual property related to Hyseq's ATP-funded project, will pursue the DNA sequencing technology upon which Hyseq was founded.

**Outlook:** Applied Biosystems has once again pledged full support for using the HyChip to develop drugs to treat genetic disorders. Once a compound starts through the Food and Drug Administration approval process for new medications, final approval is still at least eight years away. Identifying a compound to send through the approval process, however, is time-consuming. Therefore, if Hyseq and Applied Biosystems' support remains constant for their spinoff Callida, it is possible that Callida technology will impact the development of new therapeutics. Until that time, however, the outlook is uncertain.

**Composite Performance Score:** \* \* \*

**Number of Employees:** 12 employees at project start, 156 as of December 2001.

**Focus Program:** Tools for DNA Diagnostics, 1994

**Company:**

Hyseq, Inc.  
670 Almanor Avenue  
Sunnyvale, CA 94086-3513

**Contact:** Deane Little

**Phone:** (408) 524-8100